The Beagle, Records of the Museums and Art Galleries of the Northern Territory, 2008 **24**: 15–21

Jaw growth and replacement in *Diopatra aciculata* (Annelida: Onuphidae)

HANNELORE PAXTON[1] AND MILADA SAFARIK[2]

[1]*Department of Biological Sciences, Macquarie University, Sydney, NSW 2109, AUSTRALIA*
Email: hpaxton@rna.bio.mq.edu.au
[2] *Aquabait Pty Ltd, PO Box 5107, Dora Creek, NSW 2264, AUSTRALIA*

ABSTRACT

Jaw development was studied in 1- to 45-day old *Diopatra aciculata* (fertilisation to 44-chaetigers). Both mandibles and maxillae became visible in 4- to 6-chaetiger worms. The initial protomandibles become embedded in the adult mandibles. The larval maxillae consist of a left fang-like and right small serrated element, a pair of serrated plates, distal ridges, and a single carrier. Larval maxillae were replaced by juvenile maxillae in 14- to 15-chaetiger worms. Juvenile maxillae have adult-like carriers, MII and MIV, but MI are proximally dentate with a distal hook; MIII and MV are absent. First adult maxillae were seen in 21-chaetiger worms. Each subsequent moult results in larger but otherwise identical apparatuses. The moult increment (difference in size between successive moults) was established as 1.34 and used to extrapolate estimates of size classes. Based on these calculations our largest worms had their fourth set of adult maxillae, having moulted the adult maxillae every three to four days.

Keywords: Polychaeta, Eunicida, jaw apparatus, mandibles, maxillae, size classes, moulting.

INTRODUCTION

The tubicolous onuphid polychaete *Diopatra aciculata* Knox and Cameron, 1971 occurs in shallow depths along the southern shores of Australia (Paxton 1993). It is collected as prized fishing bait and commercially farmed as bait and food in the conditioning of prawn broodstock (Safarik *et al.* 2006). The worms have a lifespan of about five years (Safarik *et al.* 2006) and are dioecious. Mature eggs are about 230 μm in diameter. After a free-swimming lecithotrophic larval stage of 4 to 6 days *D. aciculata* settles, starts to build its tube and feed.

As typical for Eunicida, *D. aciculata* has a complex jaw apparatus consisting of ventral mandibles and dorsal maxillae. While mandibles grow during the lifetime of an individual, the maxillae are moulted or replaced many times. Larval and juvenile jaws have been reported for several species of Onuphidae (Table 1), although most of these reports were anecdotal. The most complete study is of *Kinbergonuphis simoni* Santos *et al.*, 1981, following the development of larval to adult maxillae and confirming the replacement of larval maxillae by adult types (Hsieh and Simon 1987), previously only known for *Mooreonuphis jonesi* Fauchald, 1982 (Fauchald 1982).

Shedding and replacement of adult maxillae in *D. aciculata,* comparable to arthropod moulting, has been reported (Paxton 2005). Replacement of maxillary jaws in Eunicida has been recently reviewed, suggesting that the type of moulting observed in Onuphidae may also take place in Eunicidae and Lumbrineridae (Paxton 2006). However, it is not known how often the maxillae are replaced. While those of older adult worms may moult infrequently or not at all, it is expected that those of young worms undergo frequent moults to keep pace with the rapidly growing body.

The aim of the present paper was to study the development of jaws in very young (1 to 45 days old) *D. aciculata* in order to describe and illustrate the growth of the mandibles and replacement of maxillae. It was hoped that relating the size of the maxillary elements to the age and size of the worms might reveal age classes and thus a possible moult cycle.

MATERIALS AND METHODS

Diopatra aciculata were reared in outside ponds at the Aquabait Pty. Ltd. aquaculture facility at Dora Creek, NSW, Australia. The study material ranged from newly fertilised eggs (day 1) to animals consisting of 44 chaetigers (maximum of 45 days). The three stages of the maxillary apparatus are referred to as larval, juvenile and adult, even though they may not correspond to the state of maturity of the animals. Animals were mounted on a slide with diluted glycerine added to the edge of the coverslip and examined with a compound microscope. The jaws were studied *in situ*. The number of chaetigers was used as an expression of body size. The elements of the adult maxillary apparatus are numbered in the conventional way in Roman numerals progressing from posterior to anterior, so that for instance MIL refers to the left most posterior maxilla, following the maxillary carrier (Fig. 1A). The length of maxilla I was taken as an indicator of the size of the complete maxillary apparatus. The maxilla I was drawn with the aid of a camera lucida and measured on the drawing.

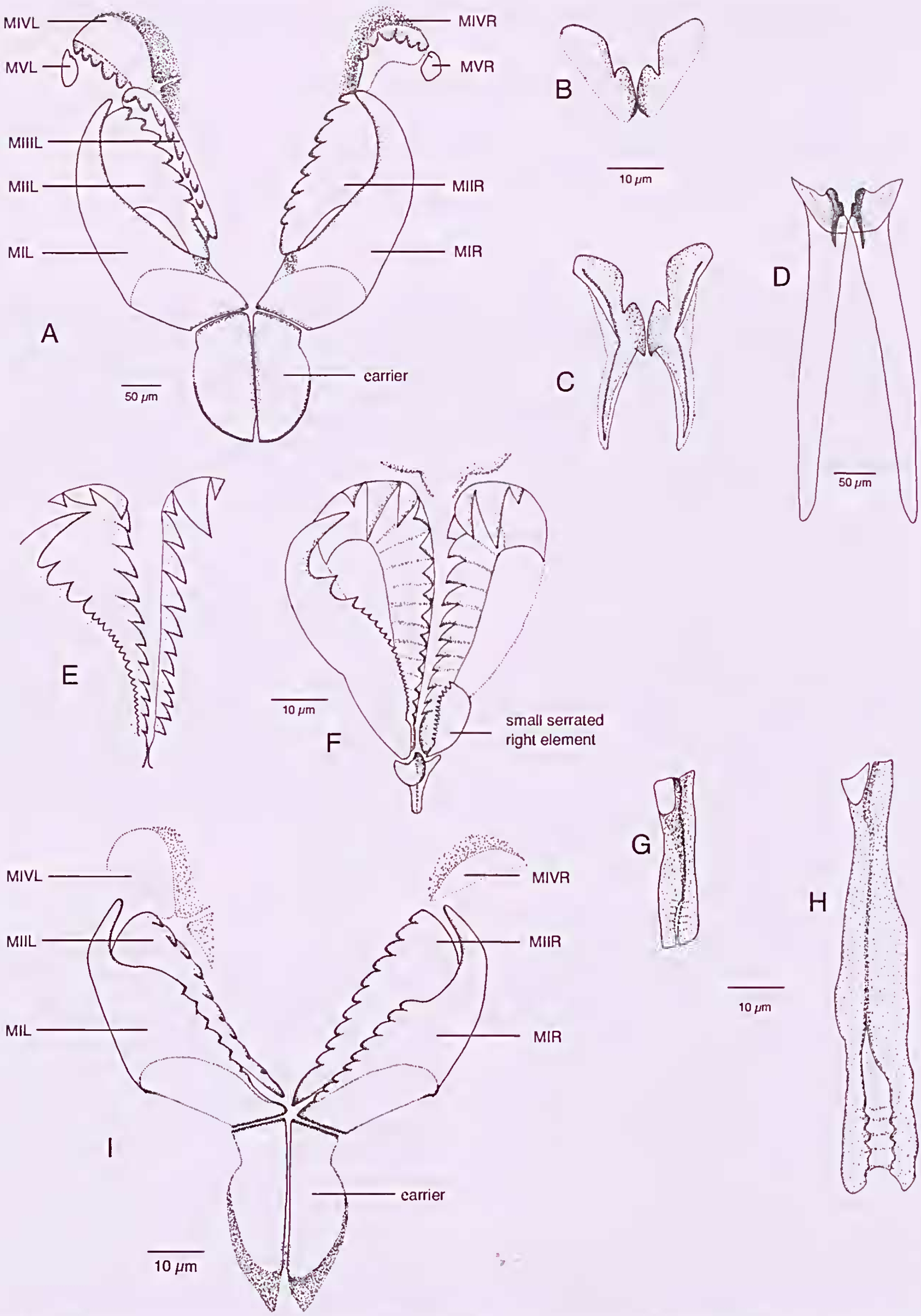

Fig. 1. Jaws of *Diopatra aciculata*: **A**, adult maxillae of 42-chaetiger specimen, dorsal view; **B**, protomandibles of 4-chaetiger specimen, dorsal view; **C**, protomandibles of 5-chaetiger specimen, dorsal view; **D**, mandibles of 42-chaetiger specimen, showing incorporated protomandibles, ventral view; **E**, developing larval maxillae in 4-chaetiger specimen, dorsal view; **F**, larval maxillae with short carrier in 4-chaetiger specimen, dorsal view; **G**, carrier of 5-chaetiger specimen, dorsal view; **H**, same of 12-chaetiger specimen, dorsal view; **I**, juvenile maxillae of 19-chaetiger specimen.

The moult increment is a term used in entomology to describe the increase in size between two successive moults. Dividing the postmoult size by the premoult size is a constant for a certain species and represents the moult increment (Gullan and Cranston 1994).

RESULTS

Overall growth. The overall growth of the worms as expressed in numbers of chaetigers over time is shown in Figure 2. The largest animals attained a size of 44 chaetigers. We are aware of the shortcomings of the quantitative nature of this data resulting from some time periods when sampling was not possible. Due to the scarcity of the material we have combined the results from two separate cohorts. While this has bolstered the numbers, it has introduced a greater range of variation due to the different growth rates of the two cohorts. However, in the absence of any previous study of this kind, we feel that the qualitative nature of the data overrides the quantitative problems.

Jaw growth – mandibles. The anterior part of the cutting plate is the first part of the mandibles to become visible in 4-chaetiger worms, when they are four to six days old. The plates initially appear as lightly sclerotised structures (Fig. 1B) and become H-like as more material is deposited (Fig. 1C). With continuous growth they develop the typical adult form which has proximal long shafts and a distal cutting plate (Fig. 1D).

Jaw replacement – maxillae. The maxillary apparatus becomes visible at the same time as the mandibles. The appearance and moulting sequence of the maxillae are summarised in Table 2.

The tips of the teeth or serrations are the first parts of the larval maxillae to become visible (Fig. 1E) and within a few days the whole apparatus is darkly sclerotised (Fig. 1F). It consists of a single narrow carrier, a large serrated left fang-like element, a very small serrated right element, a large pair of serrated plates, and a distal pair of ridges. The initial carrier is a short cup-shaped structure that articulates with the left fang-like element, has a small extension articulating with the right small plate, and a short posterior extension. This extension rapidly grows and appears to form a tube-like structure (Fig. 1G), that in some preparations opens posteriorly, displaying growth lines (Fig. 1H).

The larval maxillae are shed, swallowed, and replaced by the juvenile maxillae (Fig. 1I) in 14- to 15-chaetiger worms. That apparatus is weakly sclerotised, particularly the distal part. The juvenile maxillae consist of carriers, paired asymmetrical MI that are proximally denticulated and have a distal hook, paired denticulated MII and less distinct paired MIV. Maxillae III and V appear to be absent. The first adult maxillae (Fig. 1A) appear in 21-chaetiger worms, consisting of the typical adult form. Subsequent moults result in larger but otherwise identical maxillae.

Size classes. The length of maxilla I was taken as an indicator of the size of the total maxillary apparatus and was found to range from 80–105 μm for juvenile and

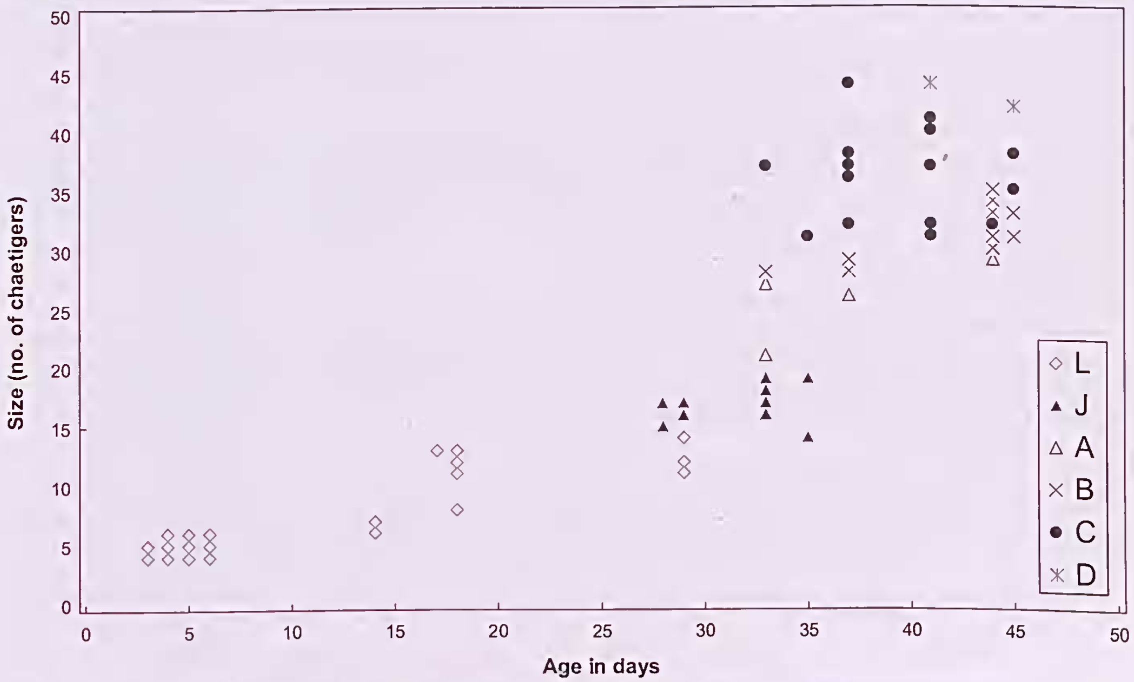

Fig. 2. Growth curve of *Diopatra aciculata* indicating stage of maxillae of each specimen, as shown in key in lower right: (**L**) larval; (**J**) juvenile; (**A**) adult stage A; (**B**) adult stage B; (**C**) adult stage C; (**D**) adult stage D.

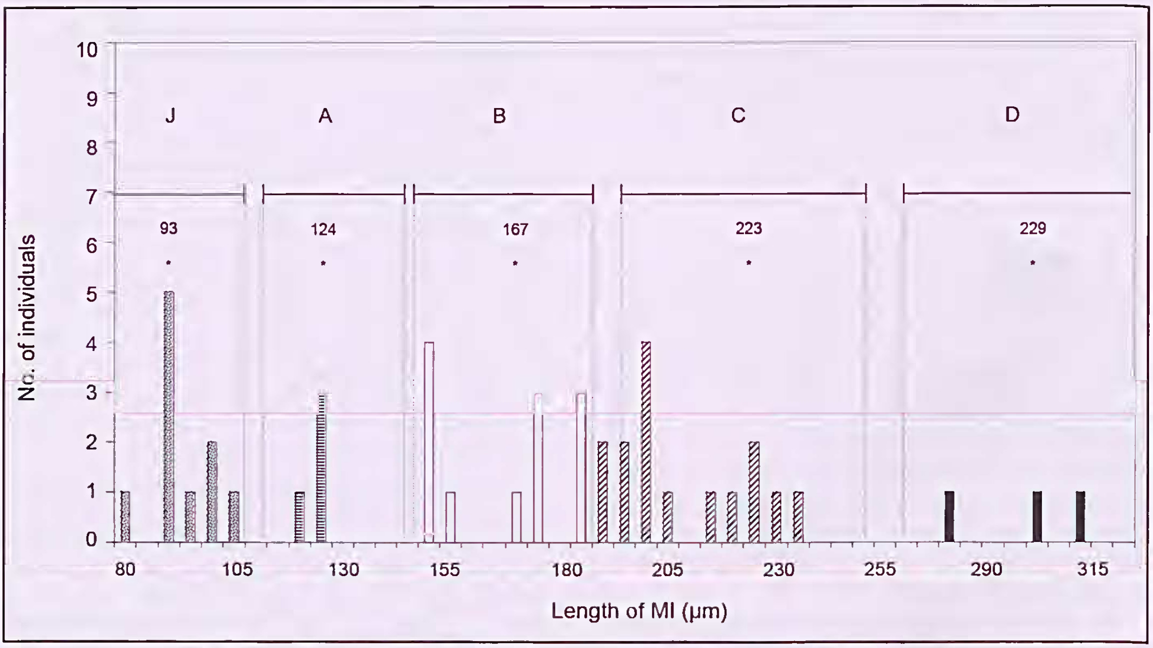

Fig. 3. Frequency histogram of MI length of juvenile (J) and adult maxillae of stages (A–D) of *Diopatra aciculata*. The mean length of each stage is indicated by asterisk and value above the respective column.

120–310 μm for adult jaws. A frequency histogram of the length of maxilla I was prepared to reveal size classes (Fig. 4). It demonstrated a well defined juvenile class. The other classes appear less clear, partly due to lack of material (class A) and continuity between classes B and C.

In the absence of clear size classes we endeavoured to discover the moult cycle utilising the moult increment (Table 3). The known moult increments came from two individuals with recently moulted maxillae *in situ* and discarded old sets recovered from the gut. A 31-chaetiger specimen (35 days old) had a moult increment of 1.32, while an 89-chaetiger specimen (82 days old) from a different study had a moult increment of 1.38. Assuming that the moult increment between the juvenile and adult maxillae equals that of consecutive adult maxillae, we calculated the value by utilising the mean size of four earliest adult and

Table 1. Reports of protomandibles, larval and juvenile maxillae for the family Onuphidae.

Species	Protomandibles Size of worm	Larval maxillae Size of worm	Juvenile maxillae Size of worm	Reference
Diopatra aciculata Knox and Cameron, 1971	4- to 20-chaetiger	same	14- to 28-chaetiger	present study
Diopatra cuprea Bosc, 1802	5- to 6-chaetiger	same	-	Allen 1959
Diopatra lilliputiana Paxton, 1993	15-chaetiger	same	-	Paxton 1993
Diopatra cf. *marocensis* Paxton *et al.*, 1995 as *D. cuprea*	11-chaetiger	same	-	Monro 1924
Hyalinoecia araucana Carrasco, 1983	13-chaetiger	same	-	Carrasco 1983
Hyalinoecia incubans Orensanz, 1990	7-chaetiger	same	-	Orensanz 1990
Kinbergonuphis simoni (Santos *et al.*, 1981)	5- to 14-chaetiger	same	15-chaetiger	Hsieh and Simon 1987
Leptoecia abyssorum Chamberlin, 1919 as *Paronuphis abyssorum*	9-chaetiger	same	-	Averincev 1972
Mooreonuphis jonesi Fauchald, 1982	-	8-chaetiger	13-chaetiger	Fauchald 1982
Onuphis elegans (Johnson, 1901)	5- and 7-chaetiger	5-chaetiger	14-chaetiger	Blake 1975

Table 2. Appearance and moulting sequence of maxillary apparatus of *D. aciculata.*

Type of maxillae	Number and type of elements in posterior to anterior order	Age and size of worm: Days since fertilisation	Number of chaetigers
Larval (Fig. 1F)	Single carrier	4 to 6	4 to 6
	Left fang-like element; small right serrated element (?MI)		
	Pair of large serrated plates (?MII)		
	Pair of ridges (?MIV)		
Juvenile (Fig. 1I)	Paired carriers	28	14 or 15
	Paired asymmetrical MI, denticulate with distal hook		
	Paired denticulated MII		
	Paired MIV		
Adult (Fig. 1A)	Paired carriers	33	21 to 29
	Paired falcate MI		
	Paired denticulate MII		
	Single left denticulate MIII		
	Paired MIV		
	Paired MV		

ten juvenile MI, establishing the value as 1.33. Thus, the available data indicate a mean moult increment of 1.34.

To obtain an estimate of size classes we used the moult increment for extrapolation. The smallest and largest juvenile MI values were multiplied by the moult increment to obtain the range of the adult (A) size class. Three more size classes were extrapolated in the same manner to give the ranges for four size classes (Table 4). The estimated size classes have been indicated for each individual in the growth curve (Fig. 2), demonstrating that the first and second moults occurred more or less at a certain chaetiger number but that the later stages overlapped. The estimated size classes were superimposed on the frequency histogram of MI length (Fig. 3), indicating again a well defined juvenile class but indistinct adult size classes.

DISCUSSION

It has been stated that the mandibles appear before the maxillae in the labidognath taxa (Kielan-Jaworowska 1966). This is not the case for *D. aciculata* and most likely other labidognath taxa as well.

While larval mandibles have been illustrated in several studies (Table 1), their relationship to the adult mandibles is generally not fully appreciated. The sclerotised larval mandibles are here referred to as protomandibles and can be noted as a dark, X-shaped structure through the body wall (Fig. 1C). They will be enlarged by the exterior deposition of sclerotised proteins and carbonates, throughout life, at the areas in contact with the cuticular epithelium, i.e. ventrally and laterally. The protomandibles are visible in young worms in a ventral view (Fig. 1D) and sometimes still visible in adults as small dark lines. In decalcified mandibles the protomandibles are sometimes longer than the sclerotised plate and have been referred to as 'Stacheln' or thorns by von Haffner (1959).

Larval maxillae are known for a number of species belonging to several genera of Onuphidae (Table 1). The main elements of the larval maxillae of all but one species were reported to consist of proximal narrow carriers, a pair of plates, a single left fang-like element and a distal pair of ridges. The only exception, *Onuphis elegans* (Johnston 1901), was reported as having paired fang-like elements (Blake 1975). The otherwise remarkable similarity of the

Table 3. Calculation of moult increment of maxillary apparatus of *D. aciculata.* *) specimen from another study (Paxton 2006)

Method	Number of worms	Stage of maxillae	Size of worms	Size of premoult MI	Size of postmoult MI	Moult increment
Observation	1	early adult	31-chaetiger	148 µm	195 µm	1.32
Observation	1	adult	89-chaetiger*	840 µm	1160 µm	1.38
Estimate	14	earliest juv./ adults	14- to 21-chaetiger	80–105, x=93 µm	120–125, x=124	1.33
					Mean of 3 values =	1.34

larval maxillary elements of *O. elegans* to those of the other species needs investigation.

The larval maxillae of *D. aciculata* (Fig. 1F) differ from those reported for other onuphids in having an additional very small serrated right element. This element is difficult to observe and presumably has been overlooked in all but one report. Krohn and Schneider (1867) reported several annelid larvae. One of these, observed at Madeira, was followed to the 5-chaetiger stage when it possessed five rudimentary prostomial appendages and the larval jaw apparatus. The authors concluded that the jaws indicated that it belonged to the family Eunicidae and because of the number of 'antennae' probably to the genus *Eunice*. We now know that *Eunice* at that stage has only one median antenna, and that the larva must have been an onuphid. The amazing part of the description and small drawing of the jaw apparatus is that Krohn observed the small right maxillary element that has presumably been overlooked by all subsequent researchers.

The larval maxillae are replaced by the juvenile maxillae (Fig. 1I). These resemble the adult apparatus with short, broad carriers, paired forceps-like MI and paired MII, and were referred to as adult jaws by Hsieh and Simon (1987). The juvenile MI differs remarkably from the adult type in that it is proximally medially dentate with a distal hook. The right hook is longer than the left one, and is similar to the prionognath type MI of the Oenonidae (e.g. *Arabella*). The apparatus is weakly sclerotised, particularly the distal part. In our specimens MI and MII were clearly visible, and MIV weakly visible. The single left MIII and paired MV of the adult maxillae appeared to be absent. However, Fauchald (1982) described and figured MIII but not MV for *Mooreonuphis jonesi* while Hsieh and Simon reported MV but not MIII for *Kinbergonuphis simoni*. We think it is probable that the MIII is not present in the juvenile apparatus and makes its first appearance in the adult apparatus. The presumed homology of the larval and juvenile maxillary elements and their evolutionary significance will be discussed elsewhere (Paxton in press).

Once the juvenile maxillae were replaced by the adult type, each additional moult resulted in slightly larger but otherwise identical maxillae. Figure 3 is a frequency histogram of maxilla I in an attempt to demonstrate size classes. A clear juvenile class was demonstrated while the other classes were inconclusive. This is not only a result of the scarcity of the data. A similar attempt to estimate size classes of the fossil polychaete *Kettnerites* (*Kettnerites*) *bankvaetensis* Bergman, 1987, was carried out by Bergman (1989). He measured 134 jaws (MI) ranging in size from 200 μm to 890 μm and did not obtain distinct size classes. Bergman discussed some factors affecting a polymodal size frequency distribution of Eunicida jaws and referred to a statement by P.J.W. Olive that with polychaetes it is virtually impossible to identify size classes beyond the second class (P.J.W. Olive pers. comm. to C. Bergman, 1986). The larger classes tended to merge due to individual growth rates, food supply and genetics.

Table 4. Estimated size classes of adult maxilla I of *D. aciculata*.

Size class	Range of size (μm)	Mean (μm)
Adult A	107–141	124
Adult B	144–189	167
Adult C	192–253	223
Adult D	258–339	299

The difference in size between two instars in insects is called the moult increment. The increase of sclerotised parts, such as the head capsule, a regular linear progression in successive instars, is known as Dyar's rule (Gullan and Cranston 1994). Our moult increment was established as 1.34 (Table 3) and is comparable to the usual insect value of 1.4 (Hinton and Mackerras 1970). Using this index, we have estimated size classes of adult maxillae (Table 4) based on the range of juvenile jaws, indicated these on Figure 3 and marked the points on Figure 2 according to the extrapolated size classes. During the 45 days of the experiment, the maxillae retained for the longest period were the larval maxillae. The worms consisted of 14–15 chaetigers (28–35 days old) at the moult to the juvenile jaws. This timing is identical with the corresponding moult in the brooding *M. jonesi* and *K. simoni*. Hsieh and Simon (1987) linked this event with leaving the maternal tube to construct tubes on their own and the commencement of feeding. Our *D. aciculata*, lecithotrophic only for a brief free-swimming larval stage, settles, starts to construct its tube and to feed at the age of 4–6 days (5-chaetigers) as evidenced by fecal pellets in the gut. This indicates that the timing of the first moult, i.e. larval to juvenile maxillae, is not governed by ecological but developmental clues.

Based on these estimations (Figs 2, 3), our largest worms (42–44 chaetigers) had their fourth set of adult maxillae, having undergone a moulting process every 3–4 days since attaining the juvenile maxillae. As the growth rate slows down, the frequency of moulting presumably slows down. Specimens from a different study (Safarik *et al.* 2006) that were raised for seven months and consisted of 150–186 chaetigers, had maxillae I of 1.6–1.9 mm length, indicating that these were their tenth adult maxillae, leading to the conclusion that the rate of moulting slows down dramatically with time, unless it occurs with less or no size increase in later life.

ACKNOWLEDGMENTS

We are grateful to Les Safarik for allowing us to use the facilities of Aquabait Pty. Ltd. for this study. We also thank Günter Purschke and Mats Eriksson for review of the manuscript and valuable suggestions.

REFERENCES

Allen, M.J. 1959. Embryological development of the polychaetous annelid, *Diopatra cuprea* (Bosc). *The Biological Bulletin of the Marine Biological Laboratory Woods Hole* **116**: 339–361.

Averincev, V.G. 1972. Benthic polychaetes Errantia from the Antarctic and Subantarctic collected by the Soviet Antarctic Expeditions. (In Russian). *Explorations of the fauna of the seas XI (XIX). Biologial Results of the Soviet Antarctic Expeditions* **5**: 88–293.

Bergman, C.F. 1989. Silurian paulinitid polychaetes from Gotland. *Fossils and Strata* **25**: 1–128.

Blake, J.A. 1975. The larval development of Polychaeta from the northern California coast. II. *Nothria elegans* (Family Onuphidae). *Ophelia* **13**: 43–61.

Carrasco, F.D. 1983. Description of adults and larvae of a new deep water species of *Hyalinoecia* (Polychaeta, Onuphidae) from the southeastern Pacific Ocean. *Journal of Natural History* **17**: 87–93.

Fauchald, K. 1982. Description of *Mooreonuphis jonesi*, a new species of onuphid polychaete from shallow water in Bermuda, with comments on variability and population ecology. *Proceedings of the Biological Society of Washington* **95**: 807–825.

Gullan, P.J. and Cranston, P.S. 1994. *The insects: an outline of entomology*. Chapman and Hall: London.

Haffner, K. von 1959. Über den Bau und den Zusammenhang der wichtigsten Organe des Kopfendes von *Hyalinoecia tubicola* Malmgren (Polychaeta, Eunicidae, Onuphidinae), mit Berücksichtigung der Gattung *Eunice*. *Zoologische Jahrbücher, Abteilung für Anatomie* **77**: 133–192.

Hinton, H.E. and Mackerras, M.J. 1970. Reproduction and metamorphosis. Pp 83–106. In: CSIRO (eds) *The insects of Australia*. Melbourne University Press: Carlton.

Hsieh, H.-L. and Simon, J.L. 1987. Larval development of *Kinbergonuphis simoni*, with a summary of development patterns in the family Onuphidae (Polychaeta). *Proceedings of the Biological Society of Washington* **7**: 194–210.

Kielan-Jaworowska, Z. 1966. Polychaete jaw apparatuses from the Ordovician and Silurian of Poland and a comparison with modern forms. *Palaeontologia Polonica* **16**: 1–152.

Knox, G.A. and Cameron, D.B. 1971. Port Philip Survey1957–1963, Victoria, Australia. Part 2, No. 4. Polychaeta. *Memoirs of the National Museum Victoria* **32**: 21–42.

Krohn, A. and Schneider, A. 1867. Ueber Annelidlarven mit porösen Hüllen. *Archiv für Anatomie, Physiologie und wissenschaftliche Medicin* **1867**: 498–508.

Monro, C.C.A. 1924. On the post-larval stages of *Diopatra cuprea*, Bosc, a polychaetous annelid of the family Eunicidae. *Annals and Magazine of Natural History (London) (9)* **14**: 193–199.

Orensanz, J.M. 1990. The Eunicemorph polychaete annelids from Antarctic and Subantarctic seas. *Biology of the Antarctic seas XXI. Antarctic Research Series* **52**: 1–183.

Paxton, H. 1993. *Diopatra* Audouin and Milne Edwards (Polychaeta: Onuphidae) from Australia, with a discussion of developmental patterns in the genus. *The Beagle, Records of the Northern Territory Museum of Arts and Sciences* **10**: 115–154.

Paxton, H. 2005. Molting polychaete jaws – ecdysozoans are not the only molting animals. *Evolution & Development* **7**: 337–340.

Paxton, H. 2006. Replacement of adult maxillary jaws in Eunicida (Polychaeta). *Scientia Marina* **70S3**: 331–336.

Paxton, H. In press. Phylogeny of Eunicida (Annelida) based on morphology of jaws. Proceedings of the 9th International Polychaete Conference.

Safarik, M., Redden, A.M. and Schreider, M.J. 2006. Density-dependent growth of the polychaete *Diopatra aciculata*. *Scientia Marina* **70S3**: 337–341.

Accepted 26 August 2008